AD_____

Award Number: DAMD17-00-1-0409

TITLE: Elevated Levels of Somatic Mutation as a Biomarker of
Environmental Effects Contributing to Breast Carcinogenesis

PRINCIPAL INVESTIGATOR: Stephen G. Grant, Ph.D.

CONTRACTING ORGANIZATION:

University of Pittsburgh
Pittsburgh, Pennsylvania   15260

REPORT DATE: July 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
                Fort Detrick, Maryland   21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
                        Distribution Unlimited

The views, opinions and/or findings contained in this report are those
of the author(s) and should not be construed as an official Department
of the Army position, policy or decision unless so designated by other
documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE July 2002	3. REPORT TYPE AND DATES COVERED Annual (1 Jul 01 – 30 Jun 02)

4. TITLE AND SUBTITLE
Elevated Levels of Somatic Mutation as a Biomarker of Environmental Effects Contributing to Breast Carcinogenesis

5. FUNDING NUMBERS
DAMD17-00-1-0409

6. AUTHOR(S)
Stephen G. Grant, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Pittsburgh
Pittsburgh, Pennsylvania 15260

E-Mail: sgg@pitt.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

20030122 059

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 Words)

It is widely presumed that environmental exposures play a role in the development of breast cancer, but few individual agents have been unequivocally identified as risk factors. Rather than seek out individual agents, we hypothesize that the cumulative effect of environmental exposures on an individual can be quantified through a blood-based assay, and further, that such a "biomarker" might distinguish breast cancer patients from age-matched controls. Preliminary evidence seems to support this hypothesis, and we have now begun to supplement this preliminary data in a manner that will allow us to determine how environmental expsoures and predisposition interact with other know risk factors for breast cancer, such as family history, life history of hormaonal exposure and exposure to ionizing radiation. These biomarker data can then be added to a risk assessment procedure for breast cancer, and ultimately, might help identify the types of exposure specifically associated with cancer in the breast.

14. SUBJECT TERMS
breast cancer, somatic mutation, GPA assay, HPRT assay, environmental exposure

15. NUMBER OF PAGES
26

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

Introduction

About 10% of incident breast cancer can be attributed to hereditary factors, and a further 15% can be accounted for by a complex mix of life history factors widely interpreted as representing endogenous hormone production. This leaves the great majority of breast cancer unaccounted for. It is assumed that environmental exposures are involved in breast carcinogenesis, and strong evidence for the effect of radiation has been presented. Total genotoxic exposure, especially the contributions of the countless chemicals in the environment, are impossible to calculate based on the agents themselves. Such exposure monitoring also does not integrate the individual response to genotoxic exposure that modulates its effect on processes such as carcinogenesis. We have proposed that direct monitoring of genetic effects at a surrogate locus in a easily available tissue can provide a biodosimter of environmental effects, and we have provided preliminary evidence that the blood-based GPA assay can provide this data. The current project is designed to expand our preliminary pool of retrospective data on 47 breast cancer patients to a more generalizable population of 200 patients, along with suitable controls for comparison. Acknowledged risk factors for breast cancer are also acquired to allow for the integration of the biomarker data into a more robust risk assessment paradigm for this disease.

Body

Risk factors for the development of breast cancer remain largely unknown, however, several clear elements have emerged: family history of breast cancer, metabolic factors related to hormone production, and exposure to X irradiation (1,2). It has been suggested that breast cancer incidence is also influenced by the accumulation of man-made chemicals in the environment. Two types of environmental chemicals have been implicated; those that mimic hormonal effects, known as "xenoestrogens", and those that mimic the DNA-damaging effects of X irradiation, or "genotoxicants". We hypothesize that breast cancer incidence should be a product of both the total cumulative exposure to genotoxic agents, including but not limited to X-rays, as modified by differences in individual response to this exposure as mediated by factors such as metabolic detoxification (or activation) and DNA repair capacity.

Although there is bound to be some element of tissue specificity for both genotoxic exposure and susceptibility to DNA damage, it is impractical to monitor somatic mutation in breast tissue itself. Blood, however, and its progenitor tissue bone marrow, are present throughout the body, and most xenobiotic exposures to the breast are likely to be transported to the breast tissue through the blood. The GPA assay is fast and inexpensive, utilizing flow technology to quickly quantify rare mutational events. However, due to its genetic basis, it can only be applied to individuals heterozygous for the MN blood group, which make up approximately 50% of the population. The HPRT assay is universally applicable, but requires cell culture and drug selection, making it more expensive and labor-intensive (3). Moreover, one class of HPRT mutants have been specifically identified as occurring via illegitimate V(D)J recombination (4), a mutagenic process that is characteristic of loss of double strand break DNA repair, such as in the cancer-prone syndrome ataxia telangiectasia (AT). The BRCA1 and BRCA 2 breast cancer

4

predisposition genes have also been implicated in this type of repair, so may also have a characteristic increase in these types of mutants (5).

This hypothesis was first applied to a mixed population of cancer patients, and these results, originally preliminary data for this proposal, have now been published (6). These data include significant contributions from cancers (breast, prostate, testicular) with acknowledged "hormonal" factors, suggesting that a dependence on genotoxic exposures is not mutually exclusive with an association with endocrine factors. We supplemented this data with analyses of local breast cancer patients, such that we had a population of 47 breast cancer patients analyzed prior to the submission of this grant. Analysis of the breast cancer patients alone confirmed that they followed the same trends as the mixed cancer population, and an odds ratio of 4.69 could be calculated for individuals with *GPA* mutation frequencies of 3×10^{-5} or higher.

Our major accomplishment of 2001 was the creation of a new IRB protocol acceptable to both our local committee and to the DOD. This was pending at the time of last report, and was approved in September, 2001. Further complications arose, however, such that final approval from the DOD to accrue samples was not granted until May 23, 2002. This delay in IRB approval is such that all personnel listed on the original submission have now left the lab; three students have completed their studies (one on another DOD-sponsored project), two lab technicians have gone on to graduate school (one to Johns Hopkins, one to Washington State) and my Clinical Coordinator cut back on her time to be home with her children. Subsequently, I only had trained personnel in the lab for about three weeks after the IRB approval came through. To make sure the patient accrual system we had set in place almost two years earlier still worked, we recruited four new patients, only one of which turned out to be informative, or heterozygous for the *GPA* assay. For practise, we ran the *HPRT* assay on all four, although in the project proper, we will only analyze patients informative at both loci (we will bank samples from *GPA* non-heterozygotes for potential future analysis with the *HPRT* assay). We are now in a period of intense recruitment and retraining, which should be completed by the beginning of the fall semester. With the recent addition of a large group of oncologists from crosstown rival Allegheny hospital, Magee-Womens Hospital and the UPMC system now treat over 700 newly diagnosed cancer patients annually; the limit on accomplishing our original goal of recruiting, sampling and analyzing 200 patients over four years is now dependent on our ability to process samples in the lab. Once again, this limitation is primarily due to the *HPRT* assay, where 50 assays a year is reasonable for a full term employee (we once ran over 700 *GPA* assays in three months, so this is not a problem). Thus, we are now in danger of falling behind our ability to finish the work on time. One solution would be to hire a clinical coordinator that also could contribute in the lab; although most such coordinators come from the ranks of nurses, Ms. Huerbin, our original coordinator, was a former lab tech and was trained in our laboratory techniques. There are several career technicians in the institute who would like a chance to become coordinators; I have evaluated several for their ability to interact with patients and our clinical colleagues. A safer route would appear to be to hire an existing clinical coordinator and accept that this project will have to be extended beyond the original target date (i.e. a no-cost extension). A final possibility is to accrue samples as quickly as possible until a total of two hundred informative patients has been achieved, then shift funding from a clinical coordinator to a second lab tech. This is possible because blood samples for HPRT analysis can be frozen and banked for analysis at a later date, apparently with no effect on mutation frequency. There is

some loss of viable cells in freezing and thawing samples, however, requiring a larger initial blood sample and yielding a higher rate of unusable samples (i.e. not enough viable cells to give a difinitive answer).

Ultimately, the path I choose will depend on the personnel avaialable. The *GPA* and *HPRT* assays are well established in my laboratory (I used the *HPRT* assay in my thesis work), and there is no reason to believe that we will not be back up to speed in a few months. In the meantime, I have published the preliminary data on a mixed population of patients given in the original submission, but in a manner that does not preclude my breaking it out in a cancer type-specific manner in a follow up. Since we have already reached statistical significance in our initial 47-patient sample for an association between *GPA* mutation frequency and cancer incidence, the present study has two main goals: to present a large enough study to persuade the reader that the results are generalizable, and b) to integrate this biomarker predictive factor into the existing models of breast cancer risk assessment. I have also used these data in the past year to propose similar studies with pediatric brain tumor patients, who share certain genetic and environmental factors with breast cancer, and to obtain funding to build upon these initial studies by analyzing breast cancer patients specifically for illegitimate VDJ recombination, a characteristic of ataxia telangiectasia patients that might be shared by BRCA1 and 2 carriers. The work proposed in this grant continues to be central to the direction of my laboratory, and despite the delays in getting started in the laboratory, I remain committed to the completion of the project and its goals

Key Research Accomplishments

o   We have completed an IRB protocol acceptable to both our local (Magee-Womens Hospital) committee and the DOD.

o   We have established that our route to patient sampling remains viable.

o   We have established that our assay technologies are still workable and can be passed on to new workers in the lab.

o   We have published our preliminary data in a form that does not preclude inclusion of data specifically on breast cancer patients in a subsequent report.

Reportable Outcomes

As the work is ongoing, all reportable outcomes are still in the future. We can confirm our preliminary data that the samples that we have run are not obviously unusual with regard to *GPA* and *HPRT* mutation frequency (as is the case with most cancer predisposition syndromes); rather we are looking at a more subtle distinction between patient and disease-free populations (approximately 1.5-fold different), consistent with the incidence of breast cancer in the "normal" population. The *GPA* assay yields some characterization of the type of mutant at the time of

analysis (the ability to separately quantify mutants arising through simple allele lose or through loss and duplication) that might provide clues as to what types of exposure and/or predisposition play a role in breast carcinogenesis at the population level and in each individual. The *HPRT* assay, through the generation of mutational spectra, can expand on this analysis; although it is beyond the span of the current study, we will be banking samples for subsequent analysis. Finally, we have already successfully funded spin-off projects that utilize additional, more specific biomarker techniques to better understand how genotoxicity plays a role in breast carcinogenesis. Thus, our eventual reportable outcomes should proceed through a confirmation that exposure biomarkers are associated with breast cancer, through an examination of how such factors interact with the known predictive factors of family history and life history of estrogen exposure, through to an examination of whether a specific type of exposure, and therefore genetic damage is implicated in breast carcinogenesis, which would allow for the development of preventive, anti-cancer agents.

Conclusions

Our data suggest that environmental exposure is a powerful factor in the development of breast cancer, but that it has too many individual contributors to monitor a single genotoxic agent. We therefore propose to monitor the effects on the individual, which has the added benefit of integrating the individual's biological modification of the initial agent and damage, or "response" at the biochemical and molecular level. With IRB concerns behind us, we are ready to get to the job of confirming and extyending our initial studies with regard to delineating the differences between those who develop breast cancer at a certain age, and those who do not.

References

1. Gail MH, Benichou J (1992) Assessing the risk of breast cancer in individuals, *in*: Cancer Prevention (DeVita VT Jr, Hellmann S, Rosenberg SA, eds), Lippincott, Philadelphia, pp. 1-15.

2. Claus EB, Risch N, Thompson WD (1994) Autosomal dominant inheritance of early-onset breast cancer. Cancer 73: 643-651.

3. Grant SG, Jensen RH (1993) Use of hematopoietic cells and markers for the detection and quantitation of human *in vivo* somatic mutation. *in*: Immunobiology of Transfusion Medicine (Garratty G, ed), Marcel Dekker, New York, pp. 299-323.

4. Fuscoe JC, Zimmerman LJ, Harrington-Brock K, Burnette L, Moore MM, Nicklas JA, O'Neill JP, Albertini RJ (1992) V(D)J recombinase-mediated deletion of the *hprt* gene in T-lymphocytes from adult humans. Mutat Res 283: 13-20.

5. Futaki M, Liu JM (2001) Chromosomal breakage syndromes and the BRCA1 genome surveillance complex. Trends Mol Med 7: 560-655.

6. Grant SG (2001) Molecular epidemiology of human cancer: biomarkers of genotoxic exposure and susceptibility. J Environ Pathol Toxicol Oncol 20: 237-253.

Appendices

Grant SG (2001) Molecular epidemiology of human cancer: biomarkers of genotoxic exposure and susceptibility. J Environ Pathol Toxicol Oncol 20: 237-253.

Journal of Environmental Pathology, Toxicology and Oncology

Volume 20/Issue 4 2001

Editor
Edgar M. Moran, M.D.

Molecular Epidemiology of Human Cancer: Biomarkers of Genotoxic Exposure and Susceptibility

Stephen G. Grant

The new field of molecular epidemiology investigates the link between toxic exposure and an associated health effect by defining presumptive intermediate stages in the development of the disease based on known mechanisms. In the development of malignancy, these steps may involve exposure to known mutagens and carcinogens, internalization and potential metabolism of a chemical agent, characterization of the interaction of the agent at its site of action (usually DNA), characterization of induced preneoplastic changes, and, in certain instances, early detection of the cancer itself. These processes can be monitored through biomarkers specific to each of the steps in the progression toward disease using any of the host of applicable techniques now available. An overview of such techniques is presented, with emphasis on techniques offering insight into the malignant process. Evidence is presented suggesting that although there are many potential contributing mechanisms to carcinogenesis, mutagenesis remains the dominant driving force behind the process. Several methods of monitoring mutation have shown promise as predictors of cancer incidence. These methods might also be used as monitors of agents designed to intervene in the process to prevent the development of overt disease.

Introduction

It has become fashionable to place the word *molecular* before the name of a classical field of scientific research and consider it reinvented. This often happens in the absence of what the word means in this context and how this refined and redefined field truly differs from its progenitor. In the case of molecular toxicology, there has been a real shift from the traditional activity of testing chemical toxicity in model systems to studies in the true organisms of interest, humans. These studies have their own advantages and disadvantages; they are, by definition, epidemiological, and epidemiology is very different from experimental science, requiring larger, more expensive and more interdisciplinary studies. Often the investigator has no control over the agent of exposure or the dose or doses administered; in most cases, one must rely on "found" experiments, such as accidental exposures, which are often uncomfortably similar to ambulance chasing. For the accumulation of significant data, more than an anecdotal case report is required, so there must be a relatively large exposed population with a consid-

Departments of Environmental and Occupation Health and Obstetrics, Gynecology and Reproductive Science, University of Pittsburgh, the University of Pittsburgh Cancer Institute and Magee-Womens Research Institute, Pittsburgh, PA. Address all correspondence to Dr. Stephen G. Grant, Department of Environmental and Occupational Health, University of Pittsburgh, 260 Kappa Drive, RIDC Park, Pittsburgh, PA 15238.

erable increase in the incidence of the disease. Indeed, this field often relies upon the pharmaceutical industry to provide a large population of exposed individuals exhibiting unanticipated toxic effects. This relative inability to study an agent of the investigator's choice is offset by the fact that real human beings are the source of data and there is no question of the applicability of the model system. These studies, therefore, involve what has become known in biomedical science as *translational* research, that is, science that has direct application to real-life situations.

In public health, the promise of molecular toxicology and molecular epidemiology is the identification of an impending disease before clinical manifestation, which potentially allows for biological, chemical, or behavioral intervention and, perhaps, prevention. This is a particularly appropriate approach to cancer because many avenues of research have shown carcinogenesis to be a multistep process with a duration or gestation time of decades. Cancer can result from the delayed effects of a single short-term exposure, such as a radiation accident, or from the effects of an otherwise asymptomatic chronic exposure. In this delayed or accumulative aspect of its etiology, it is very possible that cancer can act as a paradigm for other late-onset diseases because somatic effects are more important factors in the development of the disease than genetic predisposition. With the impending completion of the human genome project, however, attention has increasingly moved toward these genetic factors, even in diseases of aging. Besides the many technological tools being developed in this area, such as gene expression and polymorphism chips, the main reason for concentrating on genetics is that it can be fully ascertained at any age. For example, a blood sample from an 80-year-old contains all the genetic information that would have been available had the subject been sampled at birth. In contrast, toxicological exposures wax and wane, overlay one another, and are ongoing at any point of sampling; thus, there is no easily obtainable record of an exposure history similar to that of the underlying genetic background.

Molecular Epidemiology

In the classical toxicological epidemiology model, a defined health effect, often a well-characterized clin-ical disease, is perceived as occurring from the exposure of an organism to a deleterious biological, chemical, or physical agent. This strict cause-and-effect relationship is mediated through a number of unknown modifiers of exposure and response related to the anatomy, biochemistry, and physiology of the organism. The molecular epidemiological model, as shown in Figure 1, attempts to expand on the concept of such biological modification by breaking the process into sequential stages that must be traversed to manifest disease. These intermediate stages are based on mechanistic studies and hypotheses that attempt to identify the target tissue or cell type (which may not be the same as the cell type affected by the disease, or even at the same site as the eventual disease manifestation), the response or responses necessary to convert exposure into biological effect, and, if possible, the preclinical evidence of impending disease. As in any hypothetical system, experimentally verifiable predictions indicative of each stage are important. These indicators of biological modification are known as *biomarkers* and, because they precede clinical disease, they are thought of as *intermediate* biomarkers that can be used to monitor the progress of the disease.

The development of the field of molecular epidemiology has been, and continues to be, hindered by a lack of complete understanding and cooperation between the practitioners of the two progenitor disciplines, laboratory toxicologists and epidemiologists. For the toxicologist, the traditional laboratory truism, "If you need statistics to prove your point, you didn't design the experiment properly," is difficult to reconcile with epidemiological studies. On the other hand, epidemiologists, especially clinical epidemiologists, often seem to forget that statistical associations are not and cannot be proofs of causality. The proper course is for epidemiological studies to generate mechanistic hypotheses that are then evaluated experimentally. Too often, there is a complete disconnection between the two disciplines. Epidemiologists hire technicians to perform tests they have seen published in the literature, often without thoroughly understanding the relevance or implications of the results. Toxicologists, however, attempt to apply their knowledge of experimental design to epidemiological studies without appreciating the statistical methodologies necessary to adjust for unanticipated, confounding effects. In many ways,
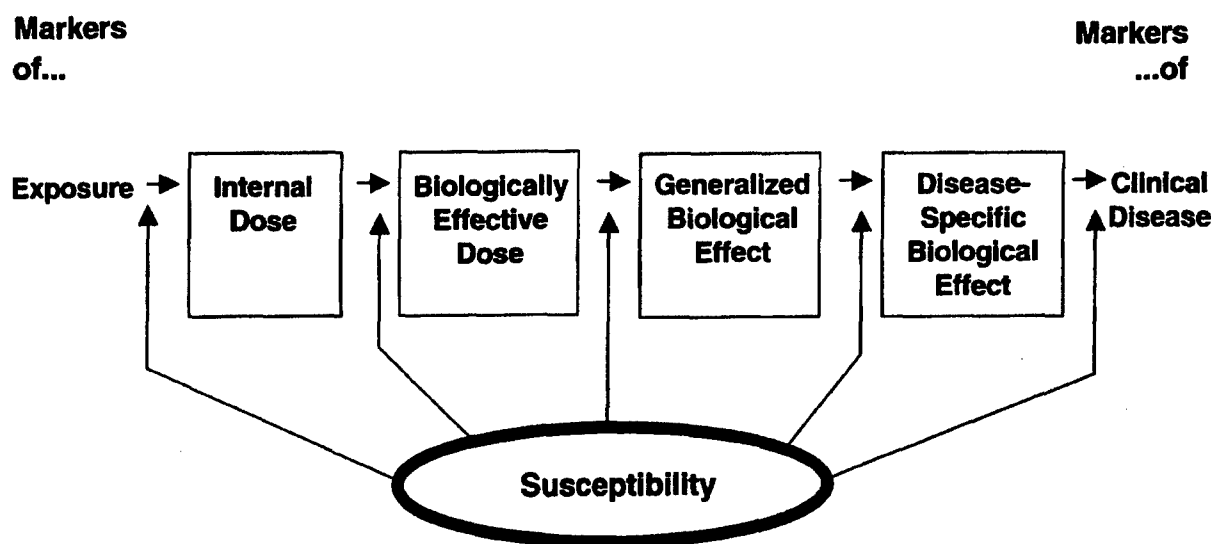
Markers of...

Markers ...of

FIGURE 1. Epidemiology of induced human disease in the mechanistic context of molecular toxicology. Insights into the absorption, distribution, metabolism, and elimination of environmental agents are combined with insights into the mechanism of the disease process to provide potential intermediate steps in the progression that can be tested for validity and applied as surrogates for the eventual health effect.[1-4]

the situation is reminiscent of the difference between academic and industrial or regulatory toxicologists: Academic toxicologists apply a continually revised or "improved" protocol to a series of individual, often unrelated projects, whereas industrial and regulatory toxicologists apply a standardized, but almost always obsolete or suboptimal protocol, to a very systematic study of an area of proven concern. Thus, in collaboration, the laboratory toxicologist can address the mechanistic relevance of a biomarker to the disease of interest, troubleshoot, and adapt the protocol to the types of samples that can be obtained, and offer the possibility of experimental follow-up on mechanistic hypotheses that might result from an epidemiological study. The epidemiologist, in turn, directs the study to a question of immediate concern to medicine or public health and allows for testing of both a mechanistic hypothesis and the biomarker designed to detect and monitor it in human studies.

Biomarkers of Carcinogenesis

To propose and test biomarkers of a specific disease, some insight into its etiology must be available. There have been many models of the carcinogenic process proposed: epigenetic, viral, toxicological, endocrine, immune surveillance, histopathological, and so forth, but the somatic mutational model[5] has become predominant for several reasons. First, there was the discovery of dominant activated oncogenes and recessive tumor suppressor genes and their identification in all types of cancer.[6,7] Second was the linking of these mutations with histological progression, as best exemplified by the Vogelstein et al.[8] model of colorectal cancer. Third, there is the unique ability (and willingness) of the supporters of this model to integrate aspects of other models into itself. For example, the somatic mutational model has to be compatible with the viral model because activated oncogenes were first identified in oncogenic viruses, and only subsequently were shown to have homologues in the host genome. The model is also flexible enough to allow that epigenetic changes in gene expression, such as endocrine stimulation, hyper- or hypomethylation of genes, can have the same effect as mutation in fulfilling the requirements of a step in the carcinogenic pathway. Toxicologists are satisfied with the mutational model because it describes a multistep process involving classical mutations that can be caused by

radiation or electrophilic chemicals. Thus, most intermediate biomarkers of cancer presume that mutation is the only or principal mechanism of carcinogenesis and are designed to detect mutagenic exposures, premutagenic, and mutagenic lesions, as well as the biological effects of somatic mutations. Toxicologists must be reminded that not all cancer researchers are prepared to directly equate carcinogenesis with mutagenesis, despite the fact that this principle underlies almost all carcinogenicity testing and costs industry billions of dollars.

Carcinogenic Exposure

Practically, there are two approaches for studying carcinogenic exposures: identification of actual exposures and identification of potential exposures. Obviously, the former is often retrospective, whereas the latter is prospective. Applied primarily to anthropogenic chemicals, a large number of carcinogenicity, mutagenicity, and other types of assays have been developed to determine or predict whether a chemical is a potential human carcinogen. The gold standard is the chronic animal cancer test; rodent carcinogenicity tests are the most widely applied.[9] These lifetime studies are time-consuming and expensive, often have questionable application to humans, and have been increasingly criticized by animal-rights activists. Attempts to establish single-cell short-term assays have usually been based on a mutational approach to the carcinogenic process, and measured genotoxicity.[10] All of these tests suffer from fundamental oversimplifications in their basic assumptions. For example, they must assume that biological effects of exposures to multiple genotoxicants (including all in vivo exposures) can be estimated from additively combining the efficacy of individual constituents, which suggests that interactions such as synergism and antagonism either do not occur, or, overall, balance one another out. They also must assume that all genotoxicants have simple dose–response kinetics, which ignores the possibilities of hormesis or other higher-order interactions. Presently, a huge number of manufactured chemicals in use lack significant toxicological data; however, there are at least two promising approaches toward im-

proving testing efficiency in the near future. First is the adoption of high-throughput and high-content screening technologies, using advances in fields such as robotics, flow cytometry, computer-directed microscopy, mass spectroscopy, and so forth, to better apply our knowledge of carcinogenesis. Such technologies have already been successfully applied in some aspects of toxicology,[11-14] but not to the degree they have been embraced by pharmacologists for drug design.[15-18] A second promising approach toward broadening our capacity to screen chemicals has also been increasingly used in pharmacological drug design: the development of so-called in silico models, or predictive-computational toxicology. Many approaches have been tried, from attempts to reproduce the logic of a working toxicologist through hierarchical sets of rules and decision trees, to correlating chemical structure or physicochemical properties with biological activity, to artificial intelligence systems such as neural networks that attempt to combine the best features of each approach.[19-21] The challenge is much greater for toxicologists than for drug designers, however, because identifying a single successful lead compound can make the approach successful for the latter, whereas missing a single toxic compound by the former could result in tragedy. Indeed, predictive models must continue to be developed through continuous interaction with traditional toxicologists, validating and extending models through targeted testing of new agents, and accounting for the greater considerations of the entire human organism and population.[22]

The second approach to defining exposures takes place in the field, often after an exposure has occurred or is suspected. Although this is the natural beginning of an epidemiological toxicological study, such physical measurements are traditionally the province of other practitioners, such as the industrial hygienist or the health physicist. Indeed, beyond the work environment or agents such as radon that are sometimes targeted by local health departments, often no attempt is made to measure or monitor the normal exposures that are thought to give rise to three-quarters of all cancer.[23] Besides the same potential problems with kinetics and interactions mentioned above, measuring genotoxicity in the field is complicated by the shear number of agents that a human being

or a population come into contact with, especially over the decades cancer may require to ensue. One approach has been to develop simple functional assays or *biosensors* that react to a spectrum of effectors rather than a single specific agent, such as a particular chemical. These instruments often use biological detectors, whole organisms or molecules such as antibodies or enzymes activated by interaction (binding) to xenobiotic agents to indicate the presence of such agents in the environment.[24] This approach is still restricted by our understanding of the underlying mechanism of action of such agents and, again, the application to cancer usually involves the assumption of a genotoxic mechanism, although methods to detect possible agents acting through an epigenetic hormonal mechanism have also been developed.[25]

Biomarkers of Exposure: Internal Dose

To manifest a carcinogenic effect, most agents must be internalized within an organism and within a cell. *Biomonitoring* of potentially toxic exposures involves measuring the agent in a tissue or bodily fluid readily available for sampling.[26] In experimental systems, a potentially toxic substance can be labeled and administered to the whole animal by various methods, and the uptake, distribution, persistence, and elimination then investigated by recovery of the label in urine and feces. In potential human exposures, similar measures can be used to infer the magnitude and importance of the original dose. Such studies are complicated by the metabolism the original agent undergoes in vivo. Indeed, if the number and types of exposures humans normally undergo are daunting, the expansion of these effects through metabolism magnifies the problem many fold. In an effort to mobilize and detoxify potentially toxic substances, the body metabolizes or *biotransforms* them into more water-soluble derivatives; unfortunately, this often makes them more reactive and, therefore, more genotoxic, also, in effect, activating them. Thus, it is usually not only the original agent that must be monitored in bodily fluids, but also a complex mixture of metabolites that have different potentials for toxicity by themselves. This metabolism of chemical agents has become an important element in the individual

exposure modification that must translate an exposure into a disease, and differences in the ability to metabolize chemicals have been shown to significantly affect their ultimate biological activity.[27] Most molecular epidemiological studies of genetic susceptibility to genotoxic agents have involved functional or genetic markers of metabolic enzymes.[28,29] Considering the number of potential phase I (esterases, cytochrome P-450 monooxygenases, epoxide hydratase, and so forth) and phase II enzymes involved in this process (methyltransferases, sulfotransferases, acetyltranferases, glucuronyl transferases, glutathione *S*-transferases, and so forth), it is difficult to predict the fate of a chemical in a biological system, although computational models have been developed.[30,31] There is an unfortunate tendency to look for associations between polymorphisms in these genes and health effects without ever determining whether the polymorphism has any effect on functionality. Since epidemiology can only be hypothesis-generating, demonstration of such an association should only provide further impetus for a functional analysis of the polymorphism and its mechanistic role in the disease process.[32,33]

Biomarkers of Exposure: Biologically Effective Dose

Genetic toxicology is a unique subspecialty of toxicology in that the target molecule, DNA, is neither cell-type- nor organ-specific. Thus, a genotoxic effect, potentially contributing to carcinogenesis, can occur in almost any cell in the body. Certain non-genotoxic carcinogenic agents, such as transforming viruses and xenoestrogens are likely to be more restricted in the types of cells they can affect. Traditionally, genotoxicants have been defined rather narrowly as agents that interact directly with the DNA, although agents affecting chromatin proteins, microtubules, and so on, can affect DNA replication and chromosome segregation. Therefore, measurement of the effective dose of a carcinogen has often been done by quantifying DNA adducts (or blood protein adducts as a surrogate). There are many methods to do this in bulk, but the most widely applied is ^{32}P-postlabeling, which yields "spots" of bases with altered migration in a two-dimensional chromatography system.[34-36]

An advantage of this and similar detection systems is that they quantitatively display all the base adduction products, so that all potential DNA damage can be estimated. The major disadvantage of such systems is that there are usually multiple species of adducted bases and, without individually characterizing each species, it is impossible to assign a relative importance to each spot. Although they must have a minimal persistence to be detectable at all (i.e., not removed from the DNA too quickly by DNA repair mechanisms), different altered bases can have very different effects on DNA replication and hydrogen bonding and, therefore, on the types and amounts of resulting mutations. Recent studies have often targeted a single, well-characterized adduction species with monoclonal antibodies; however, such studies assume that the total genotoxic effect of a mixed exposure can be estimated from a single mutagenic product, which is not likely to be consistent.[37,38]

Biomarkers of Disease: Generalized Biological Effect

In keeping with the genotoxicity paradigm for carcinogenesis, the interaction of a toxic agent with DNA does not produce a long-term effect unless it results in an unrepairable mutation, defined as any heritable change in the amount or structure of the genetic material. Since we are referring to genetic changes in somatic cells, "heritable" suggests viable clonal propagation of the mutation through subsequent mitotic generations. A large number of methods for detecting and quantifying somatic mutation have been proposed and, to some degree, validated in retrospective studies.[39] Some markers, such as micronuclei or dicentric chromosomes, are inherently inviable; they therefore serve as indicators of similar processes that leave the cell mutated but alive (a sort of biomarker of a biomarker). Other monitored events, such as sister chromatid exchange, result in no genetic damage or biological effects, but are thought to respond to agents that can, in addition, induce chromosome breakage and rearrangement. The best validated biomarker of somatic mutation is the cytogenetic detection of stable chromosome aberrations, which has been shown to be predictive of subsequent cancer in three independent prospective studies.[40–42] These studies provide strong evidence that, although other processes may contribute to human carcinogenesis, induction of somatic mutation is an important factor in cancer incidence. Measurement of gene-specific mutation has also shown promise as an intermediate biomarker of biological effect.

Somatic Mutational Analysis

There are two well-established methods for measuring gene-specific in vivo somatic mutation in humans. Both involve mutation at a non-oncogenic surrogate locus chosen to allow detection of mutation with single-hit kinetics. These well-characterized reporter genes are the X-linked gene coding for hypoxanthine-guanosine phosphoribosyl transferase (HPRT), a ubiquitously expressed purine scavenger enzyme, and the autosomal gene for erythrocyte glycophorin A (GPA), the most common sialoglycoprotein on the red cell surface, and the genetic determinant of the MN blood group. The HPRT gene has been used for many years as a selectable marker in mammalian cell culture,[43] and this assay system has been adapted to T-lymphocytes in short-term cultures derived from human peripheral blood.[44,45] The GPA assay is designed to detect a wide range of potentially inactivating mutations at the GPA locus by flow cytometric analysis of peripheral blood erythrocytes.[46,47] The two assays have complementary features (Table 1). The GPA assay is fast and inexpensive, using flow technology to quickly quantify rare mutational events. The HPRT assay requires cell culture and drug selection, making it more expensive and labor-intensive. However, the GPA assay can only be performed in genetically informative MN heterozygotes, and the mutational basis of the phenotypic variation cannot be confirmed at the molecular level, whereas the HPRT assay can be performed in virtually anyone, in a multitude of cell types, and can be used to generate mutational spectra that potentially can identify the inducing genotoxic agent. In previous studies using both assays, the correlation between these biomarkers is consistently better than the correlation of either with physical or environmental estimates of exposure, presumably because both of these assays consider the extent of exposure and the individual variations in response to genotoxic exposure.[48]

TABLE 1. Features of the GPA and HPRT In Vivo Human Somatic Mutation Assays[*]

GPA	HPRT
Well-established assay, with extensive normals database	Well-established assay, with extensive normals database
Autosomal locus sensitive to mutational, chromosomal, and epigenetic events	X-linked locus sensitive to point mutation and small deletion
Applicable to only ~50% of the population	Applicable to everyone except patients with Lesch-Nyhan syndrome
<1 mL of fresh blood required	~20 mL of fresh blood required
Inexpensive and rapid–direct-flow cytometric detection of mutants	Expensive and labor-intensive–cell culture and clonogenic drug selection
Mutant phenotype cannot be conformed at the DNA level	Mutant colonies can be genetically analyzed–generate mutational spectra

[*] Adapted from Ref. 48.

The GPA and HPRT assays have been extensively validated as quantitative measures of genotoxic exposures. Investigations include exposures to ionizing radiation such as the survivors of the bombing of Hiroshima,[49-51] accidents such as Chernobyl,[52,53] and Goiânia,[54] and other medical,[55,56] environmental,[57] and occupational studies.[58,59] Similarly, the response of these systems to chemical exposures, such as PAHs and cigarette smoke has been established in a series of studies of environmental[60,61] and occupational exposures.[62-66] Given that these assays are sensitive to a wide range of genotoxicants, it has been suggested that these measures of somatic mutation might provide a biomarker of cancer risk associated with genotoxic exposure.[67,68]

There have been three studies specifically designed to determine whether newly diagnosed cancer patients have higher somatic mutation frequencies than disease-free individuals, that is, whether cancer incidence is associated with increased levels of gene-specific (as opposed to chromosomal) mutation. In 1989, a study of lung cancer patients with the HPRT assay demonstrated significantly higher mutant frequencies in the patient population versus controls.[69] A subsequent study of breast cancer patients revealed HPRT mutant frequencies higher than controls and women with benign breast masses, but the differences failed to reach statistical significance.[70] More recently, a significant increase in mutation at the GPA locus has been reported for a population of hepatocellular carcinoma patients.[71]

Several other mutational studies of cancer patients have been performed using the GPA assay, usually to demonstrate the genotoxicity of the therapeutic regimen.[72-75] Our studies of this type have always involved analysis of both concurrent disease-free controls and a pre-therapy sample from each patient. When the results from these two populations are pooled and compared, the patients are significantly higher for total variant frequency (combining both allele-loss and loss-and-duplication classes) ($p <$ 0.01) (Fig. 2). These data include subpopulations of patients with breast,[76] prostate,[56] and testicular cancer.[77] The HPRT assay has also been used extensively to demonstrate a genotoxic effect of cancer chemotherapy upon circulating lymphocytes. In addition to the two mentioned above, seven other studies have been published in which the frequency of lymphocytes with mutations at the X-linked HPRT locus was determined in newly diagnosed cancer patients before genotoxic therapy.[78-85] In all nine studies, the frequency of somatic mutation at the HPRT locus was higher in the cancer patients than in concurrent controls. When these data were reviewed and pooled for re-analysis,[86] the approximately twofold elevation in somatic mutation frequency demonstrated by these pooled data from cancer patients (N= 187) was highly significant ($p <$ 0.001) (Fig. 3).
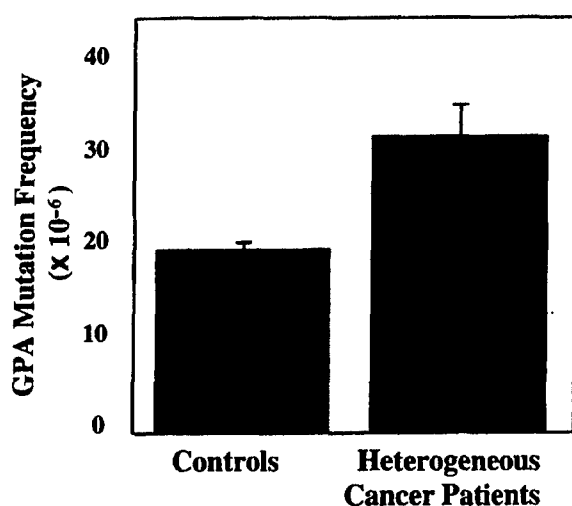
FIGURE 2. Comparison of in vivo somatic mutation at the GPA locus in a population of untreated patients with diverse types of cancer and disease-free controls.

These data suggest that human carcinogenesis is associated with increased in vivo somatic mutation and, based on the validation studies detailed above, that these mutation assays could act as integrative biodosimeters for genotoxic exposures. It is significant that the association seems to hold not just in tumors with a well-accepted mutagenic etiology, such as lung cancer, but also in tumor types with viral (hepatocarcinoma) or hormonal (breast, testicular, prostate cancer) components in their progression. This observation is consistent with the concept of a multistep mutational pathway of carcinogenicity in which one or a few steps can be fulfilled by epigenetic factors, but numerous other steps still depend on mutagenesis. These assays can measure both transient and persistent DNA damage in the stem cell and differentiating hematopoeitic compartments, respectively, and show great promise as biomonitors of chemopreventive measures against genotoxicity, such as antioxidants.

The association of cancer incidence with a modest elevation in somatic mutant frequencies suggests that cancer can be caused by normal or background levels of genotoxic exposure. Individual variation in susceptibility to genotoxic insults would therefore become an important factor in determining whether mutagenesis and, subsequently, carcinogenesis would result from a particular exposure. The HPRT and GPA

assays have also been applied to individuals and populations suffering from DNA repair-deficiency syndromes, which are characterized by very high cancer incidences. Thus, HPRT mutation has been found to be spontaneously elevated in homozygotes for the recessive cancer-prone disorders Bloom syndrome,[87] Fanconi anemia,[88,89] and ataxia telangiectasia,[90,91] all associated with deficiencies in resolving DNA double-strand breaks. The GPA assay has demonstrated 10- (ataxia telangiectasia), 50- (Fanconi anemia), and 100-fold (Bloom syndrome) increases in the frequency of spontaneous somatic mutation in these patients.[74,89,92-95] HPRT mutant frequencies appear to be elevated in xeroderma pigmentosum patients, which are characterized by a deficiency in nucleotide excision repair,[96,97] but there is no evidence for such an increase at the GPA locus.[98] Both assays have demonstrated subtle elevations in mutant frequency in the premature-aging disease, Werner syndrome.[99,100] These studies offer an alternative explanation for the elevated mutation frequencies observed in the sporadic cancer patient populations described above; namely, instead of sustaining slightly higher than normal genotoxic exposures, these individuals manifest slightly higher than normal genetic susceptibilities to genotoxic injury. This suggestion is similar
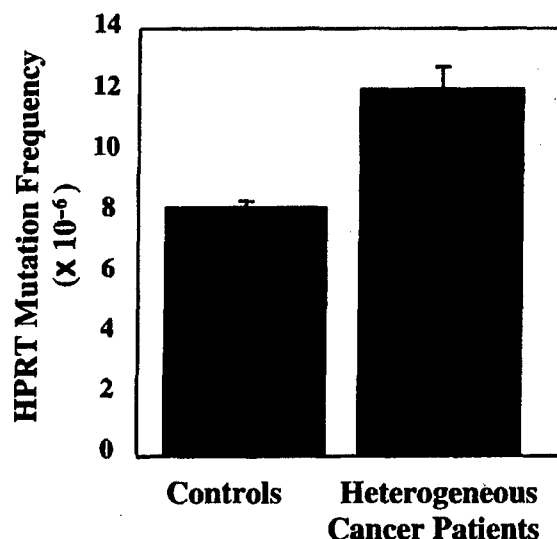

FIGURE 3. Comparison of in vivo somatic mutation at the HPRT locus in a population of untreated patients with diverse types of cancer and disease-free controls.

to the proposal by Hsu[101]: Normal populations should show interindividual variability in DNA repair capacities, and those with the highest susceptibility to unavoidable genotoxic exposures, but still within the range of normal, would be at greatest risk of developing cancer.

Mutagen Sensitivity

Hsu's own approach to demonstrating this principle was based on another characteristic of the cancer-prone syndromes: their hypersensitivity to DNA-damaging agents.[102–104] This cellular phenotype has been exploited to map and clone the underlying genes responsible for these conditions, and lymphocyte mutagen hypersensitivity continues to be used as a definitive diagnostic laboratory test. Hsu conjectured that milder forms of this mutagen-sensitivity phenotype should occur in the human population, and might contribute to the incidence of common tumors in the normal population. He adapted the mutagen-sensitivity tests developed for diagnosis of the DNA repair-deficiency diseases into a screening tool based on the induction of transient cytogenetically detectable chromatid breaks.[105–108] These studies demonstrated significant interindividual variation in the response of the disease-free population to a known genotoxic agent, the radiomimetic DNA cross-linking agent, bleomycin. They also demonstrated that a significantly greater proportion of individuals manifesting a number of different types of cancer were hypersensitive to this mutagen in that they suffered more DNA damage when their lymphocytes were exposed to a standard dose of bleomycin. This work has been carried forward by Spitz et al.,[109,110] in a series of studies demonstrating that bleomycin sensitivity is associated with risk of head and neck[109–111] and lung[112,113] cancer. Hsu et al.[114,115] introduced the idea that sensitivity to other mutagenic chemicals could also be measured by induction of chromatid breaks. In these studies, the inducing agent was 4-nitroquinoline-1-oxide (4NQO), which causes the same type of DNA damage as UV light, the genotoxic agent implicated in skin carcinogenesis. This principle has subsequently been applied in the lung cancer study using the polyaromatic hydrocarbon and tobacco smoke mutagen benzo[a]pyrene diol epoxide (BPDE) as the inducing agent.[116,117]

Biomarkers of Disease: Specific Biological Effect

Just as some would argue that an adduct is not important unless it results in a mutation, others would argue that the mutation is not important unless it is involved in the progression of the disease. Screening for mutations in oncogenes and segregation of tumor suppressor genes[118] blurs the distinctions of public health concerns, such as identifying individuals at increased risk of cancer, and purely medical concerns, that is, the early detection of the disease itself. Whatever the intent of the study, it can take the form of a screen because of the early observation that tumor cells (and potentially preneoplastic cells as well) can be found in many fluids and excreta of the body.[119] Advances in cytological techniques and the development of antibodies to cell lineage markers and carcinoembryonic antigens maintained interest in these cells, but the possibility of molecular screening only arose with the delineation of the role of somatic mutation in oncogenesis. Thus, there has been much interest and some progress in the past decade toward using molecular detection of so-called early mutations in such biological samples as buccal swabs, mouth rinses, lung lavage, urine, feces, and so on, as diagnostic and prognostic markers.[120] More recently, it has been found that free circulating DNA in serum, long known to be at higher levels in cancer patients,[121,122] is primarily derived from necrosing and apoptosing cells.[123,124] Activated oncogenes[125,126] and segregated tumor suppressor genes,[127,128] reflective of genetic changes in the primary tumor, have been detected by analysis of DNA amplified from blood samples from cancer patients.

Conclusion

In many ways, the fields of molecular toxicology and molecular epidemiology are in a holding pattern. There has been a general reluctance to leave the validation phase in which potential biomarkers are evaluated in populations with known—and usually extreme—exposures and predispositions to cancer and to move these studies into the general population, and, subsequently, into clinical or public health practice. For basic scientists, this involves taking on responsibilities for interaction with human populations

and individuals that some researchers may not appreciate. From the clinical side, in the absence of an established intervention, there may be little reason or even justification for predicting disease. Only through applying the biomarkers that do exist, such as the promising technologies discussed in this report, can basic scientists become comfortable with such translational research and can preventive measures be developed to provide practitioners with an armamentarium to treat preneoplastic disease.

The biomarkers available and in development for cancer reflect, to a large degree, the inclinations of toxicologists to equate mutagenesis with carcinogenesis. Indeed, data presented here suggest that although other mechanisms are known to contribute to cancer, mutation, both chromosomal and gene-specific, appears to be involved in all cancers. This, in turn, suggests that (1) if one lives long enough, one will inevitably develop cancer, and (2) the specific type of cancer will be that which one is most susceptible to owing to the types of exposures sustained as well as one's underlying genotype. Thus, a certain level of genotoxic effect may be sufficient to cause hepatocarcinoma in an individual with a chronic hepatitis B infection, but a slightly later onset of kidney tumor in an individual without such a viral predisposing factor. The strength of the mutational model of cancer lies in its ability to rationalize itself with these other factors. As mentioned above, we know that viruses can deliver activated oncogenes (retroviruses), or provide a protein sink for tumor suppressor gene products (animal viruses).[129] Genetic factors in cancer have been found to congenitally provide a mutation that traverses one step in the carcinogenic pathway,[130] or confer a mutator phenotype that causes a more rapid progression through the pathway,[131] or both. Hormonal factors, even if they are not overtly genotoxic, can mimic mutation via their effects on transcriptional regulation,[132] or affect mutation rates as suggested by the mitogen-mutagen hypothesis.[133] Toxicologists must also be willing to expand their definition of a mutagen; for example, because aneuploidy is unquestionably a mutational event, agents that cause it through interaction with centromeric proteins or microtubules (as opposed to direct interaction with DNA) should be considered mutagens. Despite the present success of mutationally based biomarkers, we must be aware that application of

such surrogate end points for cancer depends on the confidence the entire field feels in the underlying mechanism of cancer. Some clinicians maintain that the only credible intermediate biomarker for carcinogenesis, especially for prospective trials of chemoprevention, is the appearance of preneoplastic lesions[134]; this despite the fact that the vast majority of such lesions do not, and perhaps cannot, develop into malignant tumors.[135] Mutational biomarkers have also been criticized for not discriminating between exposure and susceptibility, or for not being more agent-specific. The best reply to such criticisms is to apply the markers we have now in the most appropriate way and, if such discrimination is found important, to continue to develop methods to further specify the relative contributions of each factor in each particular disease or lesion.

All of the preceding is based on the assumption that toxicology, pathology, and so forth, will continue playing an important role in oncology. History, however, suggests that medical science tends to follow the fad of the latest technology, even when it is not necessarily appropriate. With the recent completion of the first phase of the human genome project, we have entered into a period of increased enthusiasm for genomic research that may or may not complement the types of research discussed in this report. We mentioned earlier that every cell from 80-year-old subjects still carries their entire genetic code, facilitating such genomic research, even in such late-onset diseases as cancer. Our 80-year-olds also have a complete record of their lifetime of accumulated exposures, at least genotoxic exposures, in their cells, although different aspects may be found in different cell types, locations, and so forth. We must develop methods of rapidly screening individuals for evidence of cumulative past exposures that can be used to characterize their levels of response. The justification most often given for the extensive involvement of the U.S. Department of Energy in the human genome project was, essentially, how could we identify mutations unless we know what the normal gene sequence is? We now need to take up this challenge and use the technologies developed for charting the evolution of the hereditary genome through generations to begin to map the changes in the somatic genome that occur over a normal lifetime and during the carcinogenic process.

References

1. Hulka BS, Wilcosky T. Biological markers in epidemiologic research. Arch Environ Health 1988; 43:83–89.

2. Schulte PA. A conceptual framework for the validation and use of biologic markers. Environ Res 1989; 48:129–144.

3. Hulka BS. Epidemiological studies using biological markers: issues for epidemiologists. Cancer Epidemiol Biomarkers Prev 1991; 1:13–19.

4. Perera FP, Santella R. Carcinogenesis. In: Schulte PA, Perera FP, editors. Molecular epidemiology: principles and practices. San Diego: Academic Press, 1993: 277–300.

5. Nowell PC. The clonal evolution of tumor cell populations. Science 1976; 194:23–28.

6. Bishop JM. The molecular genetics of cancer. Science 1987; 235:305–311.

7. Green AR. Recessive mechanisms of malignancy. Br J Cancer 1988; 58:115–121.

8. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL. Genetic alterations during colorectal-tumor development. N Engl J Med 1988; 319:525–532.

9. Gold LS, Slone TH, Manley NB, Garfinkel GB, Rohrbach L, Ames BN. Carcinogenic potency database. In: Gold SL, Zeiger E, editors. Handbook of carcinogenic potency and genotoxicity databases. Boca Raton: CRC Press, 1997: 1–605.

10. Zeiger E. Genotoxicity database. In: Gold SL, Zeiger E, editors. Handbook of carcinogenic potency and genotoxicity databases. Boca Raton: CRC Press, 1997: 687–729.

11. Taylor DL, DeBiasio R, LaRocca G, Pane D, Post P, Kolega J, Giuliano K, Burton K, Gough B, Dow A, Yu J, Waggoner AS, Farkas DL. Potential of machine-vision light microscopy in toxicologic pathology. Toxicol Pathol 1994; 22:145–159.

12. Kramer PJ. Genetic toxicology. J Pharm Pharmacol 1998; 50:395–405.

13. Burchiel SW, Lauer FT, Gurule D, Mounho BJ, Salas VM. Uses and future applications of flow cytometry in immunotoxicity testing. Methods 1999; 19:28–35.

14. Van Bocxlaer JF, Clauwaert KM, Lambert WE, Deforce DL, Van den Eeckhout EG, De Leenheer AP. Liquid chromatography-mass spectrometry in forensic toxicology. Mass Spectrom Rev 2000 Aug; 19:165–214.

15. Persidis A. High-throughput screening. Advances in robotics and miniaturization continue to accelerate drug lead identification. Nat Biotech 1998; 16:488–489.

16. Silverman L, Campbell R, Broach JR. New assay technologies for high-throughput screening. Curr Opin Chem Biol 1998; 2:397–403.

17. Hopfinger AJ, Duca JS. Extraction of pharmacophore information from high-throughput screens. Curr Opin Biotechnol 2000; 11:97–103.

18. Sundberg SA. High-throughput and ultra-high-throughput screening: solution- and cell-based approaches. Curr Opin Biotechnol 2000; 11:47–53.

19. Benfenati E, Gini G. Computational predictive programs (expert systems) in toxicology. Toxicology 1997; 119:213–225.

20. Benigni R, Richard AM. Quantitative structure-based modeling applied to characterization and prediction of chemical toxicity. Methods 1998; 14:264–276.

21. Rosenkranz HS, Cunningham AR, Zhang YP, Claycamp HG, Macina OT, Sussman NB, Grant SG, Klopman G. Development, characterization and application of predictive-toxicology models. SAR QSAR Environ Res 1999; 10:277–298.

22. Holtzman S. In silico toxicology. In: Isfort RJ, Lederberg J, editors. Toxicology for the new millennium. New York: New York Academy of Sciences. Ann N Y Acad Sci 2000; 919:68–74.

23. Higginson J, Muir CS. The role of epidemiology in elucidating the importance of environmental factors in human cancer. Cancer Detect Prev 1976; 1: 79–105.

24. Schubnell D, Lehmann M, Baumann W, Rott FG, Wolf B, Beck CF. An ISFET-algal (Chlamydomonas) hybrid provides a system for eco-toxicological tests. Biosensors Bioelectron 1999; 14:465–472.

25. Seifert M, Haindl S, Hock B. In vitro analysis of xenoestrogens by enzyme linked receptor assays (ELRA). Adv Exp Med Biol 1998; 444:113–117.

26. Zielhuis RL. Biological monitoring. Scand J Work Environ Health 1978; 4:1–18.

27. Davies DS. An introduction to metabolic and kinetic aspects of toxicological studies. Xenobiotica 1988; 18 Suppl 1:3–7.

28. Autrup H. Genetic polymorphisms in human xenobiotica metabolizing enzymes as susceptibility factors in toxic response. Mutat Res 2000; 464:65–76.

29. Guengerich FP. Metabolism of chemical carcinogens. Carcinogenesis 2000; 21:345–351.

30. Ioannides C, Lewis DF, Parke DV. The use of computers in the safety evaluation of drugs and other chemicals. Eur J Drug Metab Pharmacokinet 1994; 19:225–233.

31. Klopman G, Dimayuga M, Talafous J. META. 1. A program for the evaluation of metabolic transformation of chemicals. J Chem Inform Comp Sci 1994; 34:1320–1325.

32. DuPont BR, Grant SG, Oto SH, Bigbee WL, Jensen RH, Langlois RG. Molecular characterization of glycophorin A transcripts in human erythroid cells using RT-PCR, allele-specific restriction, and sequencing. Vox Sang 1995; 68:121–129.

33. Traver RD, Siegel HD, Phillips RM, Gibson NW, Franklin WA, Ross D. Characterization of a polymorphism in NAD(P)H:quinone oxidoreductase (NQO1, DT-diaphorase). Br J Cancer 1997; 75:69–75.

34. Randerath K, Reddy MV, Gupta RC. [32P]-labeling test for DNA damage. Proc Natl Acad Sci USA 1981; 78:6126–6129.

35. International Agency for Research on Cancer (IARC). Postlabeling methods for detection of DNA adducts. In: Phillips DH, Castegnaro M, Bartsch H, editors. IARC Scientific Publication 124, Lyon: IARC, 1993.

36. International Agency for Research on Cancer (IARC). DNA adducts: identification and biological significance. In: Hemminki K, Dipple A, Shuker DEG, Kadlubar FF, Segerback D, Bartsch H, editors. IARC Scientific Publication 125, Lyon: IARC, 1994.

37. Santella RM. Immunological methods for detection of carcinogen-DNA damage in humans. Cancer Epidemiol Biomarkers Prev 1999; 8:733–739.

38. Poirier MC, Santella RM, Weston A. Carcinogen macromolecular adducts and their measurement. Carcinogenesis 2000; 21:353–359.

39. MacGregor JT, Farr S, Tucker JD, Heddle JA, Tice RR, Turteltaub KW. New molecular endpoints and methods for routine toxicity testing. Fund Appl Toxicol 1995; 26:156–173.

40. Hagmar L, Brøgger A, Hansteen I-L, Heim S, Högstedt B, Knudsen L, Lambert B, Linnainmaa K, Mitelman F, Nordenson I, Reuterwall C, Salomaa S, Skerfving S, Sorsa M. Cancer risk in humans predicted by increased levels of chromosomal aberrations in lymphocytes: Nordic study group on the health risk of chromosome damage. Cancer Res 1994; 54:2919–2922.

41. Hagmar L, Bonassi S, Stromberg U, Brøgger A, Knudsen LE, Norppa H, Reuterwall C. Chromosomal aberrations in lymphocytes predict human cancer: a report from the European study group on cytogenetic biomarkers and health. Cancer Res 1998 Sep; 58:4117–4121.

42. Bonassi S, Hagmar L, Stromberg U, Montagud AH, Tinnerberg H, Forni A, Heikkila P, Wanders S, Wilhardt P, Hansteen IL, Knudsen LE, Norppa H. Chromosomal aberrations in lymphocytes predict human cancer independently of exposure to carcinogens. Cancer Res 2000; 60:1619–1625.

43. Chu EHY, Malling HV. Mammalian cell genetics. II. Chemical induction of specific locus mutations in Chinese hamster cells in vitro. Proc Natl Acad Sci USA 1968; 61:1306–1312.

44. Strauss GH, Albertini RJ. Enumeration of 6-thioguanine-resistant peripheral blood lymphocytes in man as a potential test for somatic cell mutations arising in vivo. Mutat Res 1979; 61:353–379.

45. Morley AA, Trainor KJ, Seshadri R, Ryall RG. Measurement of in vivo mutations in human lymphocytes. Nature 1983; 302:155–156.

46. Grant SG, Bigbee WL, Langlois RG, Jensen RH. Allele loss at the human GPA locus: a model for recessive oncogenesis with potential clinical application. Clin Biotechnol 1991; 3:177–185.

47. Grant SG, Bigbee WL. In vivo somatic mutation and segregation at the human glycophorin A (GPA) locus: phenotypic variation encompassing both gene-specific and chromosomal mechanisms. Mutat Res 1993; 288:163–172.

48. Grant SG, Jensen RH. Use of hematopoietic cells and markers for the detection and quantitation of human in vivo somatic mutation. In: Garratty G, editor. Immunobiology of transfusion medicine. New York: Marcel Dekker, 1993: 299–323.

49. Langlois RG, Bigbee WL, Kyoizumi SK, Nakamura N, Bean MA, Akiyama M, Jensen RH. Evidence for increased somatic cell mutations at the glycophorin A locus in atomic bomb survivors. Science 1987; 236:445–448.

50. Langlois RG, Akiyama M, Kusunoki Y, DuPont BR, Moore DH II, Bigbee WL, Grant SG, Jensen RH. Analysis of somatic cell mutations at the glycophorin A locus in atomic bomb survivors: a comparative study of assay methods. Radiat Res 1993; 136:111–117.

51. Hakoda M, Akiyama M, Kyoizumi S, Awa AA, Yamakido M, Otake M. Increased somatic cell mutant frequency in atomic bomb survivors. Mutat Res 1988; 201:39–48.

52. Jensen RH, Langlois RG, Bigbee WL, Grant SG, Moore D II, Pilinskaya M, Vorobtsova I, Pleshanov P. Elevated frequency of glycophorin A mutations in erythrocytes from Chernobyl accident victims. Radiat Res 1995; 141:129–135.

53. Livingston GK, Jensen RH, Silberstein EB, Hinnefeld JD, Pratt G, Bigbee WL, Langlois RG, Grant SG, Shukla R. Radiobiological evaluation of immigrants from the vicinity of Chernobyl. Int J Radiat Biol 1997; 72:703–713.

54. Straume T, Langlois RG, Lucas J, Jensen RH, Bigbee WL, Ramalho AT, Brandao-Mello CE. Novel biodosimetry methods applied to victims of the Goiânia accident. Health Phys 1991; 60:71–76.

55. Nicklas JA, Falta MT, Hunter TC, O'Neill JP, Jacobson-Kram D, Williams JR, Albertini RJ. Molecular analysis of in vivo HPRT mutations in human T lymphocytes. V. Effects of total body irradiation secondary to radioimmunoglobulin therapy (RIT). Mutagenesis 1990; 5:461–468. [Erratum: Mutagenesis 1991; 6:101.]

56. Grant SG, Bigbee WL. Bone marrow somatic mutation after genotoxic cancer therapy. Lancet 1994; 343:1507–1508. [Erratum: Lancet 1994; 344:415.]

57. Bridges BA, Cole J, Arlett CF, Green MHL, Waugh APW, Beare D, Henshaw DL, Last RD. Possible association between mutant frequency in peripheral lymphocytes and domestic radon concentrations. Lancet 1991; 337:1187–1189.

58. Messing K, Ferraris J, Bradley WEC, Swartz J, Seifert AM. Mutant frequency of radiotherapy technicians appears to be associated with recent dose of ionizing radiation. Health Phys 1989; 57:537–544.

59. Straume T, Lucas JN, Tucker JD, Bigbee WL, Langlois RG. Biodosimetry for a radiation worker using multiple assays. Health Phys 1992; 62:122–130.

60. Jones IM, Moore DH, Thomas CB, Thompson CL, Strout CL, Burkhart-Schultz K. Factors affecting HPRT mutant frequency in T-lymphocytes of smokers and nonsmokers. Cancer Epidemiol Biomarkers Prev 1993; 2:249–260.

61. Ammenheuser MM, Berenson AB, Stiglich NJ, Whorton EB Jr, Ward JB Jr. Elevated frequencies of HPRT mutant lymphocytes in cigarette-smoking mothers and their newborns. Mutat Res 1994;304: 285–294.

62. Major J, Kemény G, Tompa A. Genotoxic effects of occupational exposure in the peripheral blood lymphocytes of pesticide preparing workers in Hungary. Acta Med Hung 1992–93; 49:79–90.

63. Compton-Quintana PJE, Jensen RH, Bigbee WL, Grant SG, Langlois RG, Smith MT, Rappaport SM. Use of the glycophorin A human mutation assay to study workers exposed to styrene. Environ Health Perspect 1993; 99:297–301.

64. Perera FP, Tang DL, O'Neill JP, Bigbee WL, Albertini RJ, Santella R, Ottman R, Tsai WY, Dickey C, Mooney LA, Savela K, Hemminki K. HPRT and glycophorin A mutations in foundry workers: relationship to PAH exposure and to PAH-DNA adducts. Carcinogenesis 1993; 14:969–973.

65. Dubeau H, Zazi W, Baron C, Messing K. Effects of lymphocyte subpopulations on the clonal assay of HPRT mutants: occupational exposure to cytostatic drugs. Mutat Res 1994; 321:147–157.

66. Rothman N, Haas R, Hayes RB, Li G-L, Wiemels J, Campleman S, Quintana PJE, Xi L-J, Dosemeci

N, Titenko-Holland N, Meyer KB, Lu W, Zhang LP, Bechtold W, Wang Y-Z, Kolachana P, Yin S-N, Blot W, Smith MT. Benzene induces gene-duplicating but not gene-inactivating mutations at the glycophorin A locus in exposed humans. Proc Natl Acad Sci USA 1995;92:4069–4073.

67. Albertini RJ, Nicklas JA, O'Neill JP. Somatic cell gene mutations in humans: biomarkers for genotoxicity. Environ Health Perspect 1993; 101 Suppl 3:193–201.

68. Akiyama M, Umeki S, Kusonoki Y, Kyoizumi S, Nakamura N, Mori T, Ishikawa Y, Yamakido M, Ohama K, Kodama T, Endo K, Cologne JB. Somatic-cell mutations as a possible predictor of cancer risk. Health Phys 1995; 68:643–649.

69. Tompa A, Sapi E. Detection of 6-thioguanine resistance in human peripheral blood lymphocytes (PBL) of industrial workers and lung cancer patients. Mutat Res 1989; 210:345–351.

70. Branda RF, O'Neill JP, Jacobson-Kram D, Albertini RJ. Factors influencing mutation at the HPRT locus in T-lymphocytes: studies in normal women and women with benign and malignant breast masses. Environ Mol Mutagen 1992; 19:274–281.

71. Okada S, Ishii H, Nose H, Okusaka T, Kyogoku A, Yoshimori M, Wakabayashi K. Evidence for increased somatic cell mutations in patients with hepatocellular carcinoma. Carcinogenesis 1997;18:445–449.

72. Umeki S, Kyoizumi S, Kusunoki Y, Nakamura N, Sasaki M, Mori T, Ishikawa Y, Cologne JB, Akiyama M. Flow cytometric measurements of somatic cell mutations in Thorotrast patients. Jpn J Cancer Res 1991; 82:1349–1353.

73. Hirota H, Kubota M, Adachi S, Okuda A, Lin YW, Bessho R, Wakazono Y, Matsubara K, Kuwakado K, Akiyama Y, Tsutsui T. Somatic mutations at T-cell antigen receptor and glycophorin A loci in pediatric leukemia patients following chemotherapy: comparison with HPRT locus mutation. Mutat Res 1994; 315:95–103.

74. Mott MG, Boyse J, Hewitt M, Radford M. Do mutations at the glycophorin A locus in patients treated for childhood Hodgkin's disease predict secondary leukaemia? Lancet 1994; 343:828–829.

75. Boyse J, Hewitt M, Mott MG. Glycophorin A mutations and risk of secondary leukaemia in patients treated for childhood acute lymphoblastic leukaemia. Br J Haematol 1996; 93:117–124.

76. Bigbee WL, Wyrobek AW, Langlois RG, Jensen RH, Everson RB. The effect of chemotherapy on the in vivo frequency of glycophorin A "null" variant erythrocytes. Mutat Res 1990; 240:165–175.

77. Perera FP, Motzer RJ, Tang D, Reed E, Parker R, Warburton D, O'Neill P, Albertini R, Bigbee WL, Jensen RH, Santella R, Tsai W-Y, Simon-Cereijido G, Randall C, Bosl G. Multiple biological markers in germ cell tumor patients treated with platinum-based chemotherapy. Cancer Res 1992; 52:3558–3565.

78. Albertini RJ. Drug-resistant lymphocytes in man as indicators of somatic cell mutation. Teratog Carcinog Mutagen 1980; 1:25–48.

79. Lange BJ, Prantner JE. The emergence of 6-thioguanine-resistant lymphocytes in pediatric cancer patients. Mutat Res 1982; 94:487–499.

80. Dempsey JL, Seshadri RS, Morley AA. Increased mutation frequency following treatment with cancer chemotherapy. Cancer Res 1985; 45:2873–2877.

81. Messing K, Bradley WEC. In vivo mutant frequency rises among breast cancer patients after exposure to high doses of γ-radiation. Mutat Res 1985; 152:107–112.

82. Sala-Trepat M, Cole J, Green MHL, Rigaud O, Vilcoq JR, Moustacchi E. Genotoxic effects of radiotherapy and chemotherapy on the circulating lymphocytes of breast cancer patients. III. Measurement of mutant frequency to 6-thioguanine resistance. Mutagenesis 1990; 5:593–598.

83. Ammenheuser MM, Au WW, Whorton EB Jr, Belli JA, Ward JB Jr. Comparison of HPRT variant frequencies and chromosome aberration frequencies in lymphocytes from radiotherapy and chemotherapy patients: a prospective study. Environ Mol Mutagen 1991; 18:126–135.

84. Branda RF, O'Neill JP, Sullivan LM, Albertini RJ. Factors influencing mutation at the HPRT locus in T-lymphocytes: women treated for breast cancer. Cancer Res 1991; 51:6603–6607.

85. Caggana M, Liber HL, Mauch PM, Coleman CN, Kelsey KT. In vivo somatic mutation in the lymphocytes of Hodgkin's disease patients. Environ Mol Mutagen 1991; 18:6–13.

86. Cole J, Skopek TR. Somatic mutant frequency, mutation rates and mutational spectra in the human population in vivo. Mutat Res 1994; 304:33–105.

87. Vijayalaxmi, Evans HJ, Ray JH, German J. Bloom's syndrome: evidence for an increased mutation frequency in vivo. Science 1983; 221:851–853.

88. Vijayalaxmi, Wunder E, Schroeder TM. Spontaneous 6-thioguanine-resistant lymphocytes in Fanconi anemia patients and their heterozygous parents. Hum Genet 1985; 70:264–270.

89. Sala-Trepat M, Boyse J, Richard P, Papadopoulo D, Moustacchi E. Frequencies of HPRT-lymphocytes and glycophorin A variants erythrocytes in Fanconi anemia patients, their parents and control donors. Mutat Res 1993; 289:115–126.

90. Henderson L, Cole H, Cole J, James SE, Green M. Detection of somatic mutations in man: evaluation of the microtitre cloning assay for T-lymphocytes. Mutagenesis 1986; 1:195–200.

91. Cole J, Arlett CF. Cloning efficiency and spontaneous mutant frequency in circulating T-lymphocytes in ataxia-telangiectasia patients. Int J Radiat Biol 1994; 66 Suppl 6:S123–S131.

92. Bigbee WL, Langlois RG, Swift M, Jensen RH. Evidence for an elevated frequency of in vivo somatic cell mutations in ataxia telangiectasia. Am J Hum Genet 1989; 44:402–408.

93. Langlois RG, Bigbee WL, Jensen RH, German J. Evidence for elevated in vivo mutations and somatic recombination in Bloom's syndrome. Proc Natl Acad Sci USA 1989; 86:670–674.

94. Kyoizumi S, Nakamura N, Takebe H, Tatsumi K, German J, Akiyama M. Frequency of variant erythrocytes at the glycophorin-A locus in two Bloom's syndrome patients. Mutat Res 1989; 214:215–222.

95. Grant SG, Reeger W, Wenger SL. Diagnosis of ataxia telangiectasia with the glycophorin A somatic mutation assay. Genet Test 1997–98; 1:261–267.

96. Tates AD, Bernini LF, Natarajan AT, Ploem JS, Verwoerd NP, Cole J, Green MHL, Arlett CF, Norris PN. Detection of somatic mutants in man: HPRT mutations in lymphocytes and hemoglobin mutations in erythrocytes. Mutat Res 1989; 213: 73–82.

97. Cole J, Arlett CF, Norris PG, Stephens G, Waugh APW, Beare DM, Green MHL. Elevated HPRT mutant frequency in circulating T-lymphocytes of xeroderma pigmentosum patients. Mutat Res 1992; 273:171–178.

98. Langlois RG, Bigbee WL, Jensen RH. The glycophorin A assay for somatic cell mutations in humans. In: Mendelsohn ML, Albertini RJ, editors. Mutation and the environment. Part C. Somatic and heritable mutation, adduction and epidemiology. New York: Wiley-Liss. Prog Clin Biol Res 1990; 340C: 47–56.

99. Fukuchi K-I, Tanaka K, Kumahara Y, Marumo K, Pride MB, Martin GM, Monnat RJ Jr. Increased frequency of 6-thioguanine-resistant peripheral blood lymphocytes in Werner syndrome patients. Hum Genet 1990; 84:249–252.

100. Moser MJ, Oshima J, Bigbee WL, Grant SG, Langlois RG, Jensen RH, Monnnat RJ Jr. Genetic instability and hematologic disease risk in Werner syndrome patients and heterozygotes. Cancer Res 2000; 60:2492–2496.

101. Hsu TC. Genetic instability in the human population: a working hypothesis. Hereditas 1983; 98:1–9.

102. Auerbach AD, Warburton D, Bloom AD, Chaganti RSK. Prenatal detection of the Fanconi's anemia gene by cytogenetic methods. Am J Hum Genet 1979; 31:77–81.

103. Paterson MC, Smith PJ. Ataxia telangiectasia: an inherited human disorder involving hypersensitivity to ionizing radiation and related DNA-damaging chemicals. Annu Rev Genet 1979; 13:291–318.

104. Weksberg R, Buchwald M, Sargent P, Thompson MW, Siminovitch L. Specific cellular defects in patients with Fanconi anemia. J Cell Physiol 1979; 101:311–323.

105. Cherry LM, Hsu TC. Bleomycin-induced chromosome damage in lymphocytes of medullary thyroid carcinoma patients and their family members. Anticancer Res 1983; 3:367–372.

106. Hsu TC, Cherry LM, Samaan NA. Differential mutagen susceptibility in cultured lymphocytes of normal individuals and cancer patients. Cancer Genet Cytogenet 1985; 17:307–313.

107. Hsu TC, Johnston DA, Cherry LM, Ramkissoon D, Schantz SP, Jessup JM, Winn RJ, Shirley L, Furlong C. Sensitivity to genotoxic effects of bleomycin in humans: possible relationship to environmental carcinogenesis. Int J Cancer 1989; 43:403–409.

108. Hsu TC, Spitz MR, Schantz SP. Mutagen sensitivity: a biological marker of cancer susceptibility. Cancer Epidemiol Biomarkers Prev 1991; 1:83–89.

109. Spitz MR, Fueger JJ, Beddingfield NA, Annegar JF, Hsu TC, Newell GR, Schantz SP. Chromosome sensitivity to bleomycin-induced mutagenesis: an independent risk factor for upper aerodigestive tract cancer. Cancer Res 1989; 49:4626–4628.

110. Spitz MR, Fueger JJ, Halabi S, Schantz SP, Sample D, Hsu TC. Mutagen sensitivity in upper aerodigestive tract cancer: a case-control analysis. Cancer Epidemiol Biomarkers Prev 1993; 2:329–333.

111. Cloos J, Spitz MR, Schantz SP, Hsu TC, Zhang ZF, Tobi H, Braakhuis BJ, Snow GB. Genetic susceptibility to head and neck squamous cell carcinoma. J Natl Cancer Inst 1996; 88:530–535.

112. Wu X, Hsu TC, Annegers JF, Amos CI, Fueger JJ, Spitz MR. A case-control study of nonrandom distribution of bleomycin-induced chromatid breaks in lymphocytes of lung cancer cases. Cancer Res 1995; 55:557–561.

113. Wu X, Gu J, Amos CI, Jiang H, Hong WK, Spitz MR. A parallel study of in vitro sensitivity to benzo[a]pyrene diol epoxide and bleomycin in lung carcinoma cases and controls. Cancer 1998; 83: 1118–1127.

114. Hsu TC, Feun L, Trizna Z, Savaraj N, Shirley LR, Furlong CL, Schantz SP, Weber RS, Shen TJ, Kucuk O. Differential sensitivity among three human subpopulations in response to 4-nitroquinoline-1-oxide and to bleomycin. Int J Oncol 1993; 3:827–830.

115. Hsu TC, Savaraj N, Trizna Z, Feun L, Furlong C, Schantz SP, Shirley L. Cytogenetic studies in the genotoxicity of 4-nitroquinoline-1-oxide on human cells in vitro. Int J Oncol 1993; 3:823–826.

116. Li D, Wang M, Cheng L, Spitz MR, Hittelman WN, Wei Q. In vitro induction of benzo(a)pyrene diol epoxide-DNA adducts in peripheral lymphocytes as a susceptibility marker for human lung cancer. Cancer Res 1996; 56:3638–3641.

117. Wei Q, Gu J, Cheng L, Bondy ML, Jiang H, Hong WK, Spitz MR. Benzo(a)pyrene diol epoxide-induced chromosomal aberrations and risk of lung cancer. Cancer Res 1996; 56:3975–3979.

118. Grant SG. Mutation, segregation, and childhood cancer. In: Green DM, D'Angio GJ, editors. Late effects of treatment for childhood cancer. New York: Wiley-Liss. Curr Clin Oncol 1992; 2:121–132.

119. Papanicolaou GN. A survey of the actualities and potentialities of exfoliate cytology in cancer diagnosis. Ann Intern Med 1949; 31:661–674.

120. Sidransky D. Nucleic acid-based methods for the detection of cancer. Science 1997; 278:1054–1059.

121. Leon SA, Shapiro B, Sklaroff DM, Yaros MJ. Free DNA in the serum of cancer patients and the effect of therapy. Cancer Res 1977; 37:646–650.

122. Shapiro B, Chakrabarty M, Cohn EM, Leon SA. Determination of circulating DNA levels in patients with benign or malignant gastrointestinal disease. Cancer 1983; 51:2116–2120.

123. Stroun M, Maurice P, Vasioukhin V, Lyautey J, Lederrey C, Lefort F, Rossier A, Chen XQ, Anker P. The origin and mechanism of circulating DNA. In: Anker P, Stroun M, editors. Circulating nucleic acids in plasma or serum. New York: New York Academy of Sciences. Ann N Y Acad Sci 2000; 906:161–168.

124. Jahr S, Hentze H, Englisch S, Hardt D, Fackelmayer FO, Hesch RD, Knippers R. DNA fragments in the blood plasma of cancer patients: quantitations and evidence for their origin from apoptotic and necrotic cells. Cancer Res 2001; 61:1659–1665.

125. Sorenson GD, Pribish DM, Valone FH, Memoli VA, Bzik DJ, Yao SL. Soluble normal and mutated DNA sequences from single-copy genes in human blood. Cancer Epidemiol Biomarkers Prev 1994; 3: 67–71.

126. Anker P, Lefort F, Vasioukhin V, Lyautey J, Lederrey C, Chen XQ, Stroun M, Mulcahy HE, Farthing MJ. K-ras mutations are found in DNA extracted from the plasma of patients with colorectal cancer. Gastroenterology 1997; 112:1114–1120.

127. Chen XQ, Stroun M, Magnenat JL, Nicod LP, Kurt AM, Lyautey J, Lederrey C, Anker P. Microsatellite alterations in plasma DNA of small cell lung cancer patients. Nat Med 1996; 2:1033–1035.

128. Nawroz H, Koch W, Anker P, Stroun M, Sidransky D. Microsatellite alterations in serum DNA of head and neck cancer patients. Nat Med 1996; 2:1035–1037.

129. Butel JS. Viral carcinogenesis: revelation of molecular mechanisms and etiology of human disease. Carcinogenesis 2000; 21:405–426.

130. Knudson AG. Heredity and environment in the origin of cancer. In: Klein-Szanto AJP, Anderson MW, Barrett JC, Slaga TJ, editors. Comparative

molecular carcinogenesis. New York: Wiley-Liss. Prog Clin Biol Res 1992; 376:31–40.

131. Bhattacharyya NP, Skandalis A, Ganesh A, Groden J, Meuth M. Mutator phenotypes in human colorectal carcinoma cell lines. Proc Natl Acad Sci USA 1994; 91:6319–6323.

132. Webb P, Nguyen P, Valentine C, Lopez GN, Kwok GR, McInerney E, Katzenellenbogen BS, Enmark E, Gustafsson JA, Nilsson S, Kushner PJ. The estrogen receptor enhances AP-1 activity by two distinct mechanisms with different requirements for receptor transactivation functions. Mol Endocrinol 1999; 13:1672–1685.

133. Henderson BE, Spenser Feigelson H. Hormonal carcinogenesis. Carcinogenesis 2000; 21:427–433.

134. Einspahr JG, Alberts DS, Gapstur SM, Bostick RM, Emerson SS, Gerner EW. Surrogate endpoint biomarkers as measures of colon cancer risk and their use in cancer chemoprevention trials. Cancer Epidemiol Biomarkers Prev 1997; 6:37–48.

135. Pretlow TG, Nagabhushan M, Pretlow TP. Prostatic intraepithelial neoplasia and other changes during promotion and progression. Pathol Res Prac 1995; 191:842–849.